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RPMS Technology Limited
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Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

6769301001

4. Title of the invention

IMMUNOTHERAPY

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ERIC POTTER CLARKSON
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Patents ADP number *(if you know it)*

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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11. I/We request the grant of a patent on the basis of this application.

Signature Eric Potter Clarkson Date

ERIC POTTER CLARKSON 16 January 1996

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IMMUNOTHERAPY

The present invention relates to immunotherapy, particularly to immunotherapy using cytotoxic T lymphocytes (CTL), and more particularly to adoptive immunotherapy.

There is evidence that anti-tumour CTL and anti-virus CTL play an important role *in vivo*. Tumour-reactive CTL have been shown to mediate tumour regression in animal models (1) and in man (2). Similarly, recent studies suggest that HIV-specific CTL may limit HIV virus load *in vivo* (3).

There is much interest in using *in vitro* generated CTL for adoptive immunotherapy of cancer. The potential importance of *in vitro* generated CTL is suggested in experiments with adenovirus transformed murine tumour cells (1). Nude mice were injected with tumour cells and large tumours were allowed to form. Tumour regression was observed when these mice were treated with CTL specific for the transforming E1A protein expressed in the tumour cells. Similarly, when *in vitro* generated CTL specific for gp100 were given to a melanoma patient tumour regression was observed (2). Thus, it is believed that adoptive transfer of T lymphocytes with defined specificity represents a promising therapy for cancer patients. Similarly, adoptively transferred CTL specific for cytomegalovirus seem to suppress CMV infection in patients who underwent bone marrow transplantation (4).

A major rate limiting step of current adoptive immunotherapy is that it is patient-specific and dependent upon the isolation and *in vitro* expansion of specific CTL from the patient's own T lymphocyte pool. Thus, for each patient elaborate time-consuming and expensive *in vitro* work is required

to generate sufficient numbers of specific CTL. Furthermore, in some patients the immune system may be severely suppressed, and it may be impossible to isolate specific CTL.

The present invention is aimed at overcoming these limitations and providing more efficient and potentially more effective adoptive immunotherapy with cytotoxic T lymphocytes (CTL) of patients, particularly cancer patients.

A first aspect of the invention provides a method of treating a patient with a disease wherein the patient contains diseased cells which cells contain, or are associated with, an abnormal molecule or an abnormally elevated amount of a molecule and which cells are capable of presenting at least part of said molecule on their surface by an HLA class I (or equivalent) molecule, the method comprising administering to the patient a therapeutically effective amount of cytotoxic T lymphocytes (CTL) which recognise at least part of said molecule when presented by an HLA class I (or equivalent) molecule on the surface of a cell characterised in that the cytotoxic T lymphocytes are not derived from the patient with a disease.

Thus the present invention overcomes the previous problems by, for example, generating CTL from, preferably, healthy individuals against selected peptides presented by the patient's HLA class I molecules. These CTL may be allo-restricted if the CTL donor does not express the class I molecule that presents the CTL recognised peptides, or they may be self MHC(HLA)-restricted if the CTL donor expresses the class I molecule that presents the CTL recognised peptides.

By "HLA class I (or equivalent molecule") we mean any protein which is equivalent to a human HLA class I molecule from any other animal,

particularly a vertebrate and especially a mammal. For example it is well known that in the mouse the MHC class I proteins are similar in structure to, and fulfill a similar role to, the human HLA class I proteins. Equivalent proteins to human HLA class I molecules can be readily identified in other mammalian species by a person skilled in the art, particularly using molecular biological methods.

By "at least part of said molecule" we include any fragment of said molecule that can be presented on the surface of a cell by an HLA class I (or equivalent) molecule.

By "an abnormally elevated amount of a molecule" we mean that in a diseased cell, compared to a normal cell, the molecule is present at >1.2 times the concentration; more preferably >2 times; still more preferably >5 times and most preferably >10 times the concentration.

It is particularly preferred if the CTL administered to the patient is a clonal population of CTL.

It is also particularly preferred if the CTL (preferably a clonal population of CTL) administered to the patient are substantially free of other cell types.

The molecule may be any molecule at least part of which can be presented on the surface of a cell by an HLA class I (or equivalent) molecule. Preferably, the molecule is a polypeptide including a carbohydrate-containing polypeptide such as a glycoprotein or is a carbohydrate including a peptide-containing carbohydrate.

As discussed in more detail below, abnormal molecules or an abnormally

elevated amount of a molecule are associated with many diseases and diseased cells.

It is more preferred if the said molecule is a polypeptide. It is well known in the art of immunology that peptide fragments derived by larger peptides or polypeptides are presented by HLA class I (or equivalent) molecules on the surface of a cell, especially diseased cells.

Although the CTL may be derived from the individual who is the patient from a sample taken before the patient acquired the disease, it is most preferred if the CTL are derived from an individual other than the patient.

Of course, it is preferred that the individual is a healthy individual. By "healthy individual" we mean that the individual is generally in good health, preferably has a competent immune system and, more preferably, is not suffering from any disease which can be readily tested for, and detected.

In a particularly preferred embodiment the CTL are derived from an individual which individual does not carry the HLA class I (or equivalent) molecule type which, in the patient, presents at least part of said abnormal molecule, or molecule abnormally elevated, contained in or associated with the diseased cells of said patient.

The word "type" is used in the conventional immunological sense.

Thus, the CTL are derived from an individual whose HLA class I (or equivalent) molecules are mismatched with those of the patient. Thus, it is preferred if the CTL are allo-restricted.

In this preferred embodiment the HLA class I (or equivalent) molecule types, other than the type that presents at least part of said abnormal molecule or said molecule abnormally elevated, may be the same or different as between the patient and the individual. In certain circumstances it is preferred if they are the same.

Mutant polypeptides, as are described in more detail below, are often associated with diseased cells and often serve as a molecular marker for the diseased cell. Thus, it is preferred if the polypeptide is a mutant polypeptide associated with said diseased cells.

Diseased cells, as described in more detail below, are often associated with the presence of a polypeptide at a higher level in said diseased cells compared to non-diseased cells. For example, certain polypeptides are known to be overexpressed in some tumour cells.

It is preferred if the polypeptides are any of the following:

i) normal cellular proteins that are expressed at abnormally high levels in tumours; ie cyclin D1 in a variety of tumours; cyclin E in breast cancer; mdm 2 in a variety of tumours; EGF-R, erb-B2, erb-B3, FGF-R, insulin-like growth factor receptor, Met, myc, p53 and BCL-2 in all expressed in various tumours.

ii) normal cellular proteins that are mutated in tumours; ie Ras mutations in a variety of tumours; p53 mutations in a variety of tumours; BCR/ABL translocation in CML and ALL; CSF-1 receptor mutations in AML and MDS; APC mutations in colon cancer; RET mutations in MEN2A, 2B and FMTC; EGFR mutations in gliomas; PML/RARA translocation in PML; E2A-PBX1 translocation in pre B leukaemias and in childhood acute

leukaemias.

iii) virally encoded proteins in tumours associated with viral infection; ie human papilloma virus proteins in cervical cancer; Epstein-Barr virus proteins in B cell lymphomas and Hodgkin's lymphoma; HTLV-1 proteins in adult T cell leukaemia; hepatitis B and C virus proteins in hepatocellular carcinoma; herpes-like virus proteins in Kaposi's sarcoma.

iv) HIV encoded proteins in HIV infected patients.

Thus, the antigens recognised by tumour-reactive CTL can be divided into three main categories: (i) normal self antigens expressed at high levels in tumour cells; (ii) mutated self antigens expressed in tumour cells; (iii) viral antigens expressed in tumours associated with viral infection.

Overexpression of oncogene-encoded proteins in human tumours and mutated oncogenes expressed in human tumours are described in Stauss & Dahl (1995) *Tumour Immunology*, Dalgleish/Browning, Chapter 7, incorporated herein by reference.

Thus, it is preferred if the disease to be treated is cancer; more preferably any one of breast cancer; bladder cancer; lung cancer; prostate cancer; thyroid cancer; leukaemias and lymphomas such as CML, ALL, AML, CDS, PML; colon cancer; glioma; seminoma; liver cancer; pancreatic cancer; bladder cancer; renal cancer; cervical cancer; testicular cancer; head and neck cancer; ovarian cancer; neuroblastoma and melanoma.

CML is chronic myelocytic leukaemia; ALL is acute lymphoblastic leukaemia; and AML is acute myelocytic leukaemia.

The disease to be treated may be any disease caused by a pathogen,

particularly a bacterium, yeast, virus, trypanosome and the like. It is preferred if the disease is caused by a chronic infection with a pathogen. It is also preferred if the pathogen is one which is not readily cleared by the host immune system.

It is preferred if the disease is a viral infection; more preferably a disease caused by any one of HIV, papilloma virus, Epstein-Barr virus, HTLV-1, hepatitis B virus, hepatitis C virus, herpes virus or any virus that causes chronic infection. It is particularly preferred if the virus is HIV.

Abnormal glycosylation of polypeptides is also known to occur in some diseases and diseased cells.

Abnormally elevated amounts of a hormone produced by cells occur in some diseases such as certain types of thyroid disease. Thus, the method of the invention is usefully employed to ablate the cells producing the elevated amounts of the hormone. It will be appreciated that, even if the hormone itself, or at least a part thereof, is not presented by an HLA class I (or equivalent) molecule, there may be molecules in the cell which are either abnormal or abnormally elevated and which are presented by an HLA class I (or equivalent) molecule. For example, in certain diseases where a hormone is overproduced by a cell, the biosynthetic enzymes involved in synthesis of said hormone may be overproduced by the cell.

Bacterial infections, particularly those that cause chronic infection may also be usefully treated by the method of the invention. It is preferred if the bacterial infection is an intracellular infection. Thus, the method may be useful in treating tuberculosis.

The method may also be used to treat malaria.

It is preferred if the HLA class I (or equivalent) molecule type of the patient is determined prior to administration of CTL. This is particularly preferred when the CTL are derived from an individual other than the patient whose HLA class I (or equivalent) molecules are mismatched with those of the patient.

Because of the very extensive study of the genetics of the HLA class I system the type can readily be determined using DNA typing. In particular it is convenient to use a DNA amplification-based typing system such as PCR. These methods are well known in the art and can be employed on a small tissue sample such as a saliva sample or scrape of mouth epithelial cells.

It will be appreciated that the method of the invention may be employed with any mammal such as human, cat, dog, horse, cow, sheep or pig.

It is most preferred if the patient is a human.

Although it is preferred that the patient and the donor of the CTL are the same species, for example both human, it is contemplated that the method is also useful where the patient and the donor are from different species. In other words, the method of the first aspect of the invention includes that a human patient may be given CTL from a non-human donor.

The cytotoxic T lymphocytes for use in the method of the invention, particularly a clonal population of CTL, can conveniently be made using the method of the third aspect of the invention described below.

A particularly preferred embodiment of the first aspect of the invention is wherein the HLA class I (or equivalent) molecule type of the patient is

determined prior to administration of the CTL, the CTL are derived from an individual which individual does not carry the HLA class I (or equivalent) molecule type which, in the patient, presents at least part of said abnormal molecule, or molecule abnormally, elevated contained in or associated with the diseased cells of said patient, and the CTL is selected from a library of CTL clones, said library comprising a plurality of CTL clones each derived from an individual with a different HLA class I (or equivalent) molecule type and each said CTL clone recognises said diseased cells.

More preferably each said CTL clone recognises at least part of the same molecule contained in or associated with said diseased cells.

It is preferred if between about 10^8 and 10^{11} CTL are administered to the patient; more preferably between 10^9 and 10^{10} CTL. The cells may be given to a patient who is being treated for the disease by some other method. Thus, although the method of treatment may be used alone it is desirable to use it as an adjuvant therapy.

The CTL may be administered before, during or after the other therapy.

When the disease to be treated is a cancer it is preferable if the cancer has been, is being or will be treated with a conventional therapy or surgery as well as with the method of the invention. Conveniently, depending on the therapy, the cancer is treated by radiotherapy or by chemotherapy.

When the disease to be treated is an infection by a pathogen it is preferable if the infection has been, is being or will be treated with a conventional therapy or surgery.

If the patient to be treated has HIV infection it is preferable if the method of the invention is used as an adjuvant to treatment with a reverse transcriptase inhibitor such as AZT or 3TC.

When the method of the invention is used to treat a solid tumour it is preferred if the CTL are administered as the first post-surgery treatment.

When the method of the invention is used to treat leukaemia it is preferred if the CTL are administered after radiotherapy or chemotherapy.

The CTL may be administered by any convenient route. It is preferred if the CTL are administered intravenously. It is also preferred if the CTL are administered locally to the site of the disease (such as a tumour or local viral or bacterial infection). Conveniently, the CTL are administered into an artery that supplies the site of the disease or the tissue where the disease is located.

A second aspect of the invention provides use of cytotoxic T lymphocytes (CTL) in the manufacture of a medicament for treating a patient with a disease wherein the patient contains diseased cells which cells contain, or are associated with, an abnormal molecule or an abnormally elevated amount of a molecule and which cells are capable of presenting at least part of said molecule on their surface by an HLA class I (or equivalent) molecule, wherein the cytotoxic T lymphocytes recognise at least part of said molecule when presented by an HLA class I (or equivalent) molecule on the surface of a cell and they are not derived from the patient with a disease.

A third aspect of the invention provides a method of making a clonal population of cytotoxic T lymphocytes (CTL) reactive against a selected

molecule the method comprising the step of (a) co-culturing a sample containing CTL, or a progenitor thereof, derived from a healthy individual with a stimulator cell which expresses HLA class I (or equivalent) molecules on its surface and that presents at least a part of the selected molecule in a large proportion of occupied said HLA class I (or equivalent) molecules present on the surface of said stimulator cell and (b) selecting a CTL clone reactive against said selected molecule when at least a part of said molecule is presented by an HLA class I (or equivalent) molecule on the surface of a cell.

A "sample containing CTL or a progenitor thereof" may be any suitable such sample and specifically includes, but is not limited to, peripheral blood mononuclear cells (PBMC), umbilical cord blood (which is a naive T cell source), any tissue which contains an invasion of T cells and any body fluid which contains T cells or progenitors thereof.

Preferably, said sample containing CTL or a progenitor thereof is PBMC.

Preferably, said molecule is a polypeptide.

Suitably, said selected molecule is an abnormal molecule associated with a diseased cell, or a molecule associated with a diseased cell wherein an abnormally elevated amount of said molecule is present in said diseased cell.

By "molecule associated with a diseased cell" we include any molecule which is found in an abnormal form in the diseased cell or is found in abnormally elevated levels in the diseased cells. Of course, it is most convenient if the said selected molecules, and more particularly the parts thereof presented by the HLA class I (or equivalent) molecules on the

stimulator cells, are produced outside of the said diseased cell. Methods are known, particularly computer-based methods using peptide motifs, for selecting a peptide sequence from a larger polypeptide wherein said peptide sequence is a good candidate for binding to a particular HLA class I molecule (or equivalent) type. In particular, it is preferred if said selected molecules are synthesised *in vitro*. It is particularly preferred if the part of the selected molecule is a peptide and this is made by standard peptide synthetic methods. Peptides may be synthesised by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lu *et al* (1981) *J. Org. Chem.* **46**, 3433 and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is effected using 20% piperidine in N,N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalising agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N,N-dicyclohexyl-carbodiimide/1-hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using

ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50% scavenger mix. Scavengers commonly used are ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesised. Trifluoroacetic acid is removed by evaporation *in vacuo*, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 2QJ, UK. Purification may be effected by any one, or a combination of, techniques such as size exclusion chromatography, ion-exchange chromatography and (principally) reverse-phase high performance liquid chromatography. Analysis of peptides may be carried out using thin layer chromatography, reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis.

Conveniently, said selected molecule is a mutant polypeptide associated with a diseased cell or a polypeptide present at a higher level in said diseased cell compared to a non-diseased cell.

Preferably said diseased cell is any one of a cancer cell, a virus-infected cell, a bacterium-infected cell and a cell expressing an abnormally elevated amount of a hormone.

More preferably the healthy individual is a human.

It is preferred if the polypeptides are any of the following:

i) normal cellular proteins that are expressed at abnormally high levels in tumours; ie cyclin D1 in a variety of tumours; cyclin E in breast cancer; mdm 2 in a variety of tumours; EGF-R, erb-B2, erb-B3, FGF-R, insulin-like growth factor receptor, Met, myc, p53 and BCL-2 in all expressed in various tumours.

ii) normal cellular proteins that are mutated in tumours; ie Ras mutations in a variety of tumours; p53 mutations in a variety of tumours; BCR/ABL translocation in CML and ALL; CSF-1 receptor mutations in AML and MDS; APC mutations in colon cancer; RET mutations in MEN2A, 2B and FMTC; EGFR mutations in gliomas; PML/RARA translocation in PML; E2A-PBX1 translocation in pre B leukaemias and in childhood acute leukaemias.

iii) virally encoded proteins in tumours associated with viral infection; ie human papilloma virus proteins in cervical cancer; Epstein-Barr virus proteins in B cell lymphomas and Hodgkin's lymphoma; HTLV-1 proteins in adult T cell leukaemia; hepatitis B and C virus proteins in hepatocellular carcinoma; herpes-like virus proteins in Kaposi's sarcoma.

iv) HIV encoded proteins in HIV infected patients.

In a particularly preferred embodiment the method leads to the isolation of CTL clones that recognise peptides presented by HLA class I molecules of cancer patients or HIV patients. The CTL are preferably isolated from HLA mismatched, healthy individuals. In particular, it is preferred if the healthy individual does not carry the HLA class I (or equivalent) molecule type which, on the stimulator cell, presents at least a part of the selected molecule. Once isolated, the CTL can be used for adoptive

immunotherapy of all patients expressing appropriate HLA class I molecules as described in the method of the first aspect of the invention. Conveniently, the method of this third aspect of the invention is used to generate a bank or library of CTL clones recognising peptides derived from tumour associated proteins or HIV proteins presented by different HLA class I molecules. This bank of CTL clones is available for patients expressing the appropriate HLA class I molecules. Thus, adoptive immunotherapy will no longer depend upon the elaborate production of autologous CTL clones for each patient, but will be achieved with 'ready to go' heterologous CTL clones.

The method of this aspect of the invention is particularly suited for the production of CTL against self proteins that are expressed at abnormally high levels in tumours. It is likely that cancer patients are frequently tolerant to self peptides derived from these proteins and cannot mount CTL responses. This is different in HLA mismatched individuals. Their T cell repertoire will not be tolerant to self peptides presented in the context of the class I molecules expressed by HLA mismatched cancer patients. Therefore, using HLA mismatched, healthy individuals, it will be possible to isolate CTL which recognise self peptides presented by class I molecules of cancer patients. By definition, such CTL are peptide-specific and restricted by allogeneic class I molecules. These CTL are expected to efficiently lyse tumour cells presenting high levels of these peptides, whilst the levels of peptides presented in normal cells may be insufficient for CTL lysis.

In addition to abnormally expressed self peptides, mutated self peptides derived from mutated oncogenes, or viral peptides derived from HIV also represent targets for adoptive immunotherapy. Thus, in a further preferred embodiment, CTL are generated *in vitro* from healthy

individuals. These CTL are specific for the mutated or viral peptides presented by HLA class I molecules of cancer patients or HIV infected patients. The peptide presenting class I alleles may be shared between the patients and the healthy donors, in which case the *in vitro* generated CTL will be self HLA-restricted. Alternatively and preferably, patients and healthy donors may be HLA mismatched, in which case the CTL will be allo-restricted. Allo-restricted may be advantageous in situations where the precursor frequency and/or avidity of self-restricted CTL is low.

The method of this aspect of the invention is suitable for generating allo-restricted or self-restricted CTL clones against selected peptides derived from tumour-associated proteins or HIV proteins. The CTL are conveniently generated *in vitro* by co-culturing PBMC from healthy individuals with stimulator cells that present a tumour-associated or HIV peptide in a large proportion of MHC class I molecules. This facilitates the isolation of CTL clones specific for a complex of selected peptide plus MHC class I molecule expressed by the stimulator cells. Such CTL clones may be useful for adoptive immunotherapy of all patients who express the MHC class I allele against which the CTL have been raised.

Allo-reactive CTL are of clinical relevance because they can cause transplant rejection when the MHC class I genes between donor and recipient are mismatched. In these situations, recipient CTL mount a strong immune response against the allogeneic MHC class I molecules expressed by the donor. In bone marrow transplant patients the immune response can be initiated by donor CTL against recipient MHC molecules, leading to the clinical picture of graft versus host disease (GvH). In leukaemia patients treated with bone marrow transplantation a low level of GvH is clinically favourable, since it is correlated with prolonged leukaemia free survival (5). This graft versus leukaemia (GvL) effect is

most likely due to donor CTL that can recognise and kill recipients leukaemic cells (6, 7). Whether allo-reactive CTL that mediate GvH and GvL are the same or represent distinct CTL populations has remained a controversial issue. This is because the peptide-specificity of CTL involved in GvH and GvL is generally unknown.

Although the high ligand density model postulates that allo-reactive CTL recognise allogeneic MHC molecules directly, there is currently no conclusive experimental evidence in its support. In contrast, there is good evidence that at least some allo-reactive CTL clones recognise specific peptides presented in the peptide binding groove of allogeneic MHC molecules (8, 9). Therefore, these CTL clones are peptide-specific and recognition is restricted by allogeneic class I molecules. Nevertheless, the fine specificity of primary CTL responses induced against allogeneic MHC class I molecules is usually unknown. This is because numerous peptides derived from various cellular proteins are presented in the peptide binding groove of MHC class I molecules. Thus, primary allo-restricted CTL responses are inherently poly-specific and directed against numerous MHC bound peptides of unknown sequence. This has previously made it difficult to induce allo-restricted CTL of desired peptide specificity. In addition to this technical difficulty, the possibility of inducing peptide-specific, allo-restricted CTL previously has not been seriously investigated because it violates a fundamental immunological concept. The selection of the T cell repertoire takes place in the thymus where two key events occur (10). During negative selection T cells expressing T cell receptors (TCRs) that recognise with high affinity MHC molecules presenting self peptides are deleted from the repertoire. In contrast, TCRs that recognise MHC/peptide complexes with low affinity are positively selected and released into the periphery as mature T cells. It is believed that as a consequence of positive selection the mature T cells are self MHC-

restricted. Thus, mature T cells are thought to efficiently recognise immunogenic peptides only when they are presented by self MHC molecules, but not when they are presented by allogeneic MHC molecules.

Here, it is proposed to employ allo-restricted as well as self-restricted CTL from healthy individuals for adoptive immunotherapy. The CTL recognised peptides may be derived from proteins that are overexpressed in tumours, from mutated proteins, or from viral proteins. In experiments described below, we found that it is possible to isolated peptide-specific, allo-restricted CTL. Some allo-restricted CTL clones can recognise very low concentrations of peptides (femtomolar concentrations) indicating that they are at least as sensitive (perhaps even more sensitive) than self-restricted CTL which typically require picomolar peptide concentrations for recognition. We also found that these CTL can be injected three times into immunocompetent hosts without causing any immunological reactions (eg anaphylaxis or hypersensitivity). The allo-restricted CTL clones are probably most efficient for short term treatment of immunocompromised patients. It is unlikely that these CTL will have any long term side effects because they will be eventually eliminated by a functional host immune response.

A particularly preferred embodiment is the generation of allo-restricted CTL against known epitopes in HIV proteins and in tumour-associated proteins. A number of CTL recognised peptides have been identified in various HIV proteins and in tumour-associated proteins. In particular, CTL epitopes have been identified in the HIV *env*, *gag*, *pol*, *vif* and *nef* proteins (12, 13). Also, CTL epitopes have been identified in the tumour-associated melanoma proteins tyrosinase, mart1/melanA, gp100/pmel17, mage and bage (14-21). The use of peptides corresponding to these CTL

epitopes has the advantage that they are known to be produced by natural antigen processing. CTL produced in this way recognise target cells expressing the relevant proteins endogenously. The exploitation of known CTL epitopes represents a considerable shortcut because it avoids screening of large numbers of test peptides and identification of naturally produced peptides. However, known peptides may represent immunodominant peptides. The method of the third aspect of the invention may be used to identify new peptides, which new peptides may be preferred as they are likely to be subdominant peptides. Since subdominant peptides are less likely to be immunoselected by patient's CTL responses, they may represent better targets for adoptive immunotherapy. Nevertheless, peptides representing known CTL epitopes can be ideally exploited to generate allo-restricted or self-restricted CTL *in vitro* and to test their anti-viral and anti-tumour effects *in vivo*.

The method of the third aspect of the invention allows the isolation of HLA class I-restricted CTL clones specific for peptides produced in tumour cells and for peptides produced in HIV infected cells. Conveniently, SCID mouse models are used to determine the *in vivo* anti-tumour and anti-HIV effects of these CTL. These CTL clones are useful for adoptive immunotherapy, especially in humans.

It is preferred if the method of the third aspect of the invention further comprises determining the HLA class I (or equivalent) molecule type of the healthy individual. Conveniently, this is done by DNA analysis as disclosed above.

It is particularly preferred if the stimulator cell has a type of HLA class I (or equivalent) molecule on its surface which HLA class I (or equivalent) molecule type is not present in the healthy individual.

It is particularly preferred if said stimulator cell is a cell which is substantially incapable of itself loading said HLA class I (or equivalent) molecule with at least a part of said selected molecule. As is described in more detail below, the HLA class I (or equivalent) molecule may readily be loaded with at least a part of said selected molecule *in vitro*.

Conveniently, said cell is a mammalian cell defective in the expression of a peptide transporter such that, when at least part of said selected molecule is a peptide, it is not loaded into said HLA class I (or equivalent) molecule.

Preferably the mammalian cell lacks or has a reduced level of the TAP peptide transporter. Suitable cells which lack the TAP peptide transporter include T2, RMA-S and *Drosophila* cells. TAP is the Transporter Associated with antigen Processing.

Thus, conveniently the cell is an insect cell such as a *Drosophila* cell.

The human peptide loading deficient cell line T2 is available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA under Catalogue No CRL 1992; the *Drosophila* cell line Schneider line 2 is available from the ATCC under Catalogue No CRL 19863; the mouse RMA-S cell line is described in Karre and Ljunggren (1985) *J. Exp. Med.* **162**, 1745, incorporated herein by reference.

In a preferred embodiment the stimulator cell is a host cell (such as a T2, RMA-S or *Drosophila* cell) transfected with a nucleic acid molecule capable of expressing said HLA class I (or equivalent) molecule. Although T2 and RMA-S cells do express before transfection HLA class

I molecules they are not loaded with a peptide.

Mammalian cells can be transfected by methods well known in the art. *Drosophila* cells can be transfected, as described in Jackson *et al* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 12117, incorporated herein by reference.

Conveniently said host cell before transfection expression expresses substantially no HLA class I (or equivalent) molecules.

It is also preferred if the stimulator cell expresses a molecule important for T cell costimulation such as any of B7.1, B7.2, ICAM-1 and LFA 3.

The nucleic acid sequences of numerous HLA class I (and equivalent) molecules, and of the costimulator molecules, are publicly available from the GenBank and EMBL databases.

It is particularly preferred if substantially all said HLA class I (or equivalent) molecules expressed in the surface of said stimulator cell are of the same type.

HLA class I in humans, and equivalent systems in other animals, are genetically very complex. For example, there are at least 110 alleles of the HLA-B locus and at least 90 alleles of the HLA-A locus. Although any HLA class I (or equivalent) molecule is useful in this aspect of the invention, it is preferred if the stimulator cell presents at least part of the selected molecule in an HLA class I molecule which occurs at a reasonably high frequency in the human population. It is well known that the frequency of HLA class I alleles varies between different ethnic groupings such as Caucasian, African, Chinese and so on. At least as far as the Caucasian population is concerned it is preferred that HLA class I

molecule is encoded by an HLA-A2 allele, or an HLA-A1 allele or an HLA-A3 allele or an HLA-B7 allele. HLA-A2.1 is particularly preferred.

When the method of the third aspect of the invention is used to make a library of CTL it is convenient if the CTL clones in the library are selected on the basis of frequency in a particular ethnic grouping.

It will be appreciated that a stimulator cell which expresses HLA class I (or equivalent) molecules on its surface and that presents at least a part of a selected molecule in a large proportion of occupied said HLA class I (or equivalent) molecules present on the surface of said stimulator cell forms a further aspect of the invention.

Preferably the selected molecule is an abnormal molecule or a molecule whose amount is abnormally elevated.

A fourth aspect of the invention provides a clonal population of cytotoxic T lymphocytes reactive against a selected molecule obtainable by the method of the third aspect of the invention.

A fifth aspect of the invention provides a clonal population of cytotoxic T lymphocytes reactive against a selected molecule wherein the said CTL has a high avidity for a cell presenting said selected molecule in a HLA class I (or equivalent) molecule.

It will be appreciated that, at least for self molecules abnormally elevated, and in particular for self polypeptides expressed at high levels, the method of the third aspect of the invention allows the production of CTL of much higher avidity and sensitivity than can otherwise be produced. This is particularly the case when the stimulator cell has a type of HLA class I (or

equivalent) molecule on its surface which HLA class I (or equivalent) molecule type is not present in the healthy individual. Thus, for a given peptide and a given HLA class I (or equivalent) molecule, the CTL of this aspect of the invention have a high avidity. The avidity can be measured by standard titration methods using the CTL and the peptide. Allo restricted CTL require lower peptide concentrations for recognition.

Thus, the method of the third aspect of the invention is preferably used to produce cytotoxic T lymphocytes (CTL) from healthy individuals that can be used for adoptive immunotherapy of cancer patients and patients infected with the human immunodeficiency virus. The CTL are generated entirely *in vitro* and may be administered to patients intravenously. Since this form of adoptive immunotherapy does not depend upon a functional host immune system, it is believed to be particularly suited to patients who are immunosuppressed, for example as a consequence of HIV infection or radiotherapy and chemotherapy in the case of cancer. Preferably, all peptide-specific CTL are isolated from healthy donors, and no blood samples from patients are required.

A sixth aspect of the invention provides a clonal population of cytotoxic T lymphocytes according to the fourth or fifth aspects of the invention for use in medicine.

A seventh aspect of the invention provides a pharmaceutical composition comprising clonal population of cytotoxic T lymphocytes according to the fourth or fifth aspects of the invention and a pharmaceutically acceptable carrier.

The aforementioned CTL of the invention or a formulation thereof may be administered by any conventional method including by parenteral (eg

subcutaneous or intramuscular) injection. The treatment may consist of a single dose or a plurality of doses over a period of time.

Whilst it is possible for the CTL of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

An eighth aspect of the invention provides use of a clonal population of cytotoxic T lymphocytes derived from a healthy individual and reactive against a selected abnormal molecule derived from a diseased cell from a patient with a disease, or a selected molecule derived from a diseased cell from a patient with a disease wherein an abnormally elevated amount of said molecule is present in said diseased cell, in the manufacture of a medicament for treating a patient with the disease wherein said healthy individual has a different HLA type to said patient.

A ninth aspect of the invention provides a library of CTL clones, said library comprising a plurality of CTL clones derived from individuals with differing HLA class I (or equivalent) molecule type and each said CTL clone recognises a molecule associated with a selected disease.

The library is conveniently stored in a form where each CTL clone retains viability. Conveniently the library is stored frozen.

Preferably, the library contains a selection of CTLs which have been made by the method of the third aspect of the invention. The library may be disease or disease cell specific or it may be HLA class I (or equivalent)

molecule type specific. Preferred diseases or HLA class I (or equivalent) molecule types are described above.

A tenth aspect of the invention provides a therapeutic system comprising (a) means to determine the HLA class I (or equivalent) type of a patient to be treated and (b) a library of CTL clones, said library comprising a plurality of CTL clones derived from individuals with differing HLA class I (or equivalent) molecule type and each said CTL clone recognises a molecule associated with a selected disease.

The invention will now be described in more detail with reference to the following Examples and Figures wherein:

Figure 1 shows the results of an experiment where mice were injected with $5x10^5$ RMA tumour cells only or with tumour cells and $5x10^5$ CTL. The tumour volume was measured every day. After 11 days mice that received tumour cells only were killed because of tumour ulceration or because of large tumour burden. None of the mice that received CTL had detectable tumours at day 11.

Figure 2 shows the results of an experiment where mice were injected with $5x10^5$ RMA cells at day 7, and at day 0 they were treated with 10^7 anti-mdm100 CTL i.v. The tumour volume was measured at day 0 and each subsequent day. The relative increase in tumour volume is shown. Mice were killed when tumours ulcerated or reached more than 3 cm^3 in volume.

Example 1: Adoptive immunotherapy using CTL in mice

In a murine model system we have generated allo-restricted CTL clones

against peptides derived from the normal self protein mdm 2 which is frequently overexpressed in tumours. The CTL kill tumour cells *in vitro*, whilst normal cells are not recognised. When adoptively transferred into mice, these CTL show anti-tumour effects *in vivo*.

The two mouse strains C57BL/10 (H-2^b) and BALB/c (H-2^d) are MHC mismatched and therefore express distinct class I molecules. This MHC mismatch was chosen because it mimics the HLA mismatch found in the human situation, ie the difference between a cancer patient and a healthy T cell donor. We used these two mouse strains to test whether allo-restricted CTL against the murine mdm 2 protein can be detected. The mdm 2 protein can associate with p53 and regulate its biological activity. It is frequently overexpressed in human cancers and consequently represents a possible target for adoptive immunotherapy.

In the murine model system described above, we explored the following questions:

(i) Is it possible to isolate mdm 2 peptide-specific, H-2K^b-restricted CTL from BALB/c mice (H-2^d haplotype)?

(ii) Can the BALB/c derived peptide-specific, allo-restricted CTL recognise tumour cells expressing K^b class I molecules?

(iii) Can the BALB/c derived peptide-specific, allo-restricted CTL be used for adoptive tumour immunotherapy in C57BL/10 mice (H-2^b haplotype)?

We have obtained the following results:

(i) CTL that are specific for an mdm 2 peptide presented by the allogeneic H-2K^b class I molecule were isolated.

(ii) CTL clones were established and shown to kill RMA thymoma cells of H-2^b origin. RMA cells are highly tumourigenic in C57BL/10 mice (H-2^b) which allowed us to test whether BALB/c derived CTL might have anti-tumour effects in C57BL/10 hosts.

(iii) In one experiment 8 mice were injected s.c. with $5x10^5$ RMA cells. 4 of these mice were also injected with $5x10^5$ CTL. After 11 days, the 4 mice that had been injected with RMA cells alone, had large tumours and were therefore killed, whilst the 4 mice that had received RMA and CTL were tumour free (Fig 1). In another experiment, 8 mice were injected s.c. with $5x10^5$ tumour cells. After 7 days, when all the mice had developed tumours at the site of injection, 2 of them were treated i.v. with 10^7 BALB/c derived CTL. In the untreated mice the tumour volume increased rapidly, whilst in those that received CTL, the tumour growth was delayed (Fig 2).

These results show that this adoptive immunotherapy approach using cytotoxic T cells is effective.

Example 2: Identification of CTL epitopes in HIV proteins and in tumour-associated proteins

In order to serve as CTL targets peptides have to (i) be able to bind to HLA class I molecules; (ii) be able to stimulate CTL; (iii) be produced by natural antigen processing. These three requirements are tested experimentally.

(i) *Identification of peptides in HIV proteins or in tumour-associated proteins which bind to HLA class I molecules*

Peptide binding motifs have been identified for a large number of HLA class I molecules (11). These binding motifs are used to screen the sequences of HIV proteins or tumour-associated proteins. Motif-containing peptides are synthesised and the binding to the appropriated class I alleles is analysed. To analyse peptide binding, HLA class I molecules are expressed in the peptide loading mutant cell line T2 of human origin. T2 cells express naturally HLA-A2.1 class I molecules. The expression of other HLA class I molecules (eg A1, B7 etc) is achieved by transfecting corresponding class I genes into T2 cells. Since T2 cells have a peptide loading defect, they express only low levels of peptide-containing class I molecules on their cell surface. However, if class I binding peptides are present in the culture medium, the levels of MHC class I expression are enhanced. The enhanced levels of class I expression is detected by staining T2 cells with HLA class I-specific antibodies, followed by analysis with a fluorescence activated cell sorter. Peptide-titration experiments reveal the efficiency of class I binding of individual peptides.

As an alternative to the described peptide-binding assay using intact T2 cells, binding assays are performed in cell lysates. Lysates of metabolically labelled T2 cells are incubated overnight at 4°C in the presence of test peptides. Antibodies specific for conformationally correctly folded HLA class I molecules are used for immunoprecipitation. Only few conformationally folded class I molecules are detectable when T2 cell lysates are incubated in the absence of class I binding peptides. In contrast, if test peptides bind to class I molecules, this will stabilise their conformation. Consequently, increased levels of radiolabelled class I molecules are immunoprecipitated from T2 cell lysates incubated with class I binding peptides.

Radioactively labelled MHC class I molecules are not necessarily required to measure peptide binding. For example, known class I binding peptides can be labelled by iodination or by biotinylation and serve as indicator peptides in competition experiments. Thus, lysates from T2 cells containing HLA class I molecules are incubated with labelled indicator peptides together with varying concentrations of unlabelled test peptides. Test peptides which bind to HLA class I molecules successfully inhibit binding of indicator peptides. This results in a reduction of HLA class I molecules containing labelled indicator peptides. Thus, the amount of labelled indicator peptides detectable by immunoprecipitation with antibodies specific for conformationally correctly folded class I molecules is decreased.

T2 cells are not the only source of HLA class I molecules for binding assays. *Drosophila* cells represent an alternative source. *Drosophila* cells are transfected with human $\beta 2$ microglobulin in conjunction with genes encoding various HLA class I alleles. Since *Drosophila* cells do not contain the TAP proteins required for peptide transport and MHC peptide loading, the transfected class I alleles are properly loaded with peptides. Thus, the conformation of class I molecules in lysates of transfected *Drosophila* cells is unstable in the absence of HLA binding peptides. Binding assays in lysates of *Drosophila* cells are performed under the same conditions as described for lysates prepared from T2 cells.

(ii) *Stimulation of peptide-specific, allo-restricted CTL*

Test peptides which bind efficiently to HLA class I molecules (see above) are used to stimulate CTL responses from healthy, HLA unrelated individuals. Allo-reactive CTL responses against peptides other than the test peptides are avoided. The probability of stimulating CTL against test

peptides is increased if the majority of HLA molecules expressed by the stimulator cells have test peptides in their binding groove. Thus, the approach is to express by transfection the same HLA class I molecules in various human and non-human cells, and to load them with class I binding test peptides. These peptide loaded cells are then used to stimulate CTL from PBMC of HLA unrelated healthy individuals. The PBMC are stimulated every 1-2 weeks with different human and non-human cell types expressing the same HLA molecule. This decreases the probability of stimulating CTL against irrelevant peptides because the different human and non-human cell types most likely present different sets of these irrelevant peptides.

The following cell types are suitable for expression of HLA class I molecules and for CTL stimulation: the human cell lines T2 and C1R, the mouse cell lines RMA, RMA-S and P1HTR, and *Drosophila* cells that were transfected to express not only HLA class I molecules but also molecules that are important for T cell costimulation such as B7.1, B7.2, ICAM1 and LFA3.

To achieve high levels of MHC occupancy with test peptides the HLA transfected T2, RMA-S and *Drosophila* cells are particularly suitable. These cell types do not express functional TAP peptide transporter molecules and consequently express a large proportion of peptide deficient MHC molecules which can be loaded with exogenously added peptides. Thus, these cells are incubated overnight with 100 μM of test peptides and then used to stimulate CTL using PBMC of healthy donors. To achieve high levels of MHC occupancy with test peptides in the normal cell lines C1R, RMA and P1HTR, these cells are treated with a buffer containing 0.2 M acetic acid and 0.2 M sodium chloride at pH 3-4 for approximately 1 minute to denature the peptide containing MHC class I molecules on the

surface of these cells. The cells are then incubated at neutral pH in medium containing human β2-microglobulin and 100-200 μM of test peptides. During this incubation, a large proportion of HLA class I molecules refold and contain the test peptide in their binding groove.

CTL cultures are initiated in 24 well plates using $5x10^6$ responder PBMC obtained from buffy coat blood packs and 10^6 irradiated, peptide-loaded stimulator cells per well. The culture medium consists of RPMI, 10% FCS, 10% culture supernatant of anti-CD4 monoclonal antibodies and 10 U/ml recombinant IL-2. After one week the responding CTL are restimulated in micro-cultures in 96 well plates. Each well contains $2x10^5$ irradiated autologous PBMC feeder cells and 10^5 irradiated peptide-loaded stimulator cells. Varying numbers of responder CTL starting from approximately $5x10^4$ to $5x10^2$ are seeded per micro-culture. Replica cultures of 24 wells are set up for each responder cell number. The micro-cultures are restimulated with fresh irradiated PBMC and peptide-loaded stimulator cells after 10-14 days. 7 days after the last stimulation, the CTL from individual micro-cultures are analysed in a ^{51}Cr release assay against T2 cells expressing the appropriate HLA-class I molecules presenting the test peptides that are used to generate the CTL or presenting irrelevant control peptides. Micro-cultures that show preferential killing of T2 cells coated with test peptides over T2 cells coated with irrelevant peptides are expanded to confirm the CTL specificity. At the same time, some of these CTL are used for limiting dilution cloning on 96 well plates using the same numbers of feeder cells and stimulator cells as for the micro-cultures described above. 1 to 0.5 CTL are seeded per well. The specificity of CTL clones is confirmed using T2 target cells coated with relevant and irrelevant peptides.

(iii) *Identification of peptides produced by natural antigen processing*

Whether test peptides that bind well to HLA class I molecules ((i) above) and that stimulate CTL responses ((ii) above) are produced by natural antigen processing is determined. Initially, peptides are selected by screening the sequences of HIV proteins and of tumour-associated proteins for the presence of MHC class I binding motifs. The work described in sections (i)-(ii) above shows that selected test peptides can bind to class I molecules and can stimulate peptide-specific CTL. Whether natural antigen processing leads to the MHC class I presentation of these peptides is determined. Peptide-specific CTL lines and clones are tested against target cells expressing relevant HIV proteins or tumour-associated proteins endogenously. Thus, human C1R cells are doubly transfected with the relevant HLA class I genes and the genes encoding HIV proteins or tumour-associated protein. CTL lysis of doubly transfected C1R target cells in the absence of lysis of singly transfected cells shows that natural processing of endogenously expressed proteins produces the peptides recognised by these CTL clones.

Example 3: Adoptive immunotherapy using CTL in humans

Similar *in vitro* stimulation conditions to those described in Example 2 for the mouse produce human allo-restricted CTL. Allo-restricted CTL recognising peptides derived from HIV proteins or from tumour-associated proteins presented by the HLA-A2.1 class I molecule are generated. (Allo-restricted CTL recognising peptides presented by HLA-A1 and HLA-B7 will be isolated may also be used.) These three HLA alleles are among the most frequent in Caucasian populations and there is a high probability that any one individual will express at least one of the three alleles. This means, that CTL clones restricted by these three HLA alleles is useful for adoptive immunotherapy of the majority of individuals of a Caucasian population. The CTL clones are used as an adjuvant to

chemotherapy in treating the cancer.

For adoptive immunotherapy of HIV infected individuals, peptides derived from HIV encoded proteins are exploited. Virus load is suppressed following administration of the CTL.

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CLAIMS

1. A method of treating a patient with a disease wherein the patient contains diseased cells which cells contain, or are associated with, an abnormal molecule or an abnormally elevated amount of a molecule and which cells are capable of presenting at least part of said molecule on their surface by an HLA class I (or equivalent) molecule, the method comprising administering to the patient a therapeutically effective amount of cytotoxic T lymphocytes (CTL) which recognise at least part of said molecule when presented by an HLA class I (or equivalent) molecule on the surface of a cell characterised in that the cytotoxic T lymphocytes are not derived from the patient with a disease.

2. A method according to Claim 1 wherein the CTL are a clonal population of CTL.

3. A method according to Claim 1 or 2 wherein the CTL are substantially free of other cell types.

4. A method according to any one of Claims 1 to 3 wherein said molecule is a polypeptide.

5. A method according to any one of Claims 1 to 4 wherein the CTL are derived from an individual other than the patient.

6. A method according to any one of Claims 1 to 5 wherein the CTL are derived from an individual which individual does not carry the HLA class I (or equivalent) molecule type which, in the patient, presents at least part of said abnormal molecule, or molecule

abnormally elevated, contained in or associated with the diseased cells of said patient.

7. A method according to Claim 4 wherein said polypeptide is a mutant polypeptide associated with said diseased cells.

8. A method according to Claim 4 wherein said polypeptide is present at a higher level in said diseased cells compared to non-diseased cells.

9. A method according to any one of the preceding claims wherein the disease is a cancer.

10. A method according to Claim 9 wherein the cancer is any one of any one of breast cancer; bladder cancer; lung cancer; prostate cancer; thyroid cancer; leukaemias and lymphomas such as CML, ALL, AML, CDS, PML; colon cancer; glioma; seminoma; liver cancer; pancreatic cancer; bladder cancer; renal cancer; cervical cancer; testicular cancer; head and neck cancer; ovarian cancer; neuroblastoma and melanoma.

11. A method according to any one of Claims 1 to 8 wherein the disease is caused by a chronic viral infection.

12. A method according to Claim 11 wherein the virus is any one of HIV, papilloma virus, Epstein-Barr virus, HTLV-1, hepatitis B virus, hepatitis C virus and herpes virus.

13. A method according to Claim 12 wherein the virus is HIV.

14. A method according to any one of Claims 1 to 8 wherein the disease is associated with an abnormally elevated amount of a hormone.

15. A method according to any one of Claims 1 to 8 wherein the disease is a bacterial disease caused by a chronic bacterial infection.

16. A method according to any one of the preceding claims further comprising the step of determining the HLA class I (or equivalent) molecule type of the patient prior to administration of the CTL.

17. A method according to Claim 16 wherein the said type is determined using DNA typing.

18. A method according to any one of the preceding claims wherein the patient is human.

19. A method according to Claim 16 when dependent on Claim 6 wherein said cytotoxic T lymphocyte is selected from a library of CTL clones, said library comprising a plurality of CTL clones derived from individuals with differing HLA class I (or equivalent) molecule type and each said CTL clone recognises said diseased cells.

20. A method according to Claim 19 wherein each said CTL clone recognises at least part of the same molecule contained in or associated with said diseased cells.

21. Use of cytotoxic T lymphocytes in the manufacture of a

medicament for treating a patient with a disease wherein the patient contains diseased cells which cells contain, or are associated with, an abnormal molecule or an abnormally elevated amount of a molecule and are capable of presenting at least part of said molecule on their surface by an HLA class I (or equivalent) molecule, wherein the cytotoxic T lymphocytes recognise at least part of said molecule when presented by an HLA class I (or equivalent) molecule on the surface of a cell and they are not derived from the patient with a disease.

22. A method of making a clonal population of cytotoxic T lymphocytes (CTL) reactive against a selected molecule the method comprising the step of (a) co-culturing a sample containing CTL or a progenitor, thereof derived from a healthy individual with a stimulator cell which expresses HLA class I (or equivalent) molecules on its surface and that presents at least a part of the selected molecule in a large proportion of occupied said HLA class I (or equivalent) molecules present on the surface of said stimulator cell and (b) selecting a CTL clone reactive against said selected molecule when at least a part of said molecule is presented by an HLA class I (or equivalent) molecule on the surface of a cell.

23. A method according to Claim 22 wherein the healthy individual does not carry the HLA class I (or equivalent) molecule type which, on the stimulator cell, presents at least a part of the selected molecule.

24. A method according to Claim 22 or 23 wherein said sample containing CTL or a progenitor thereof is PBMC.

25. A method according to any one of Claims 22 to 24 wherein said molecule is a polypeptide.

26. A method according to any one of Claims 22 to 25 wherein said selected molecule is an abnormal molecule associated with a diseased cell, or a molecule associated with a diseased cell wherein an abnormally elevated amount of said molecule is present in said diseased cell.

27. A method according to Claim 26 wherein the said selected molecule is a mutant polypeptide associated with a diseased cell or a polypeptide present at a higher level in said diseased cell compound to a non-diseased cell.

28. A method according to Claim 26 or 27 wherein said diseased cell is any one of a cancer cell, a virus-infected cell, a bacterium infected cell and a cell expressing an abnormally elevated amount of a hormone.

29. A method according to any one of Claims 22 to 28 wherein the healthy individual is a human.

30. A method according to Claim 29 wherein the said selected molecule is any one of cyclin D1, cyclin E, mdm 2, EGF-R, erb-B2, erb-B3, FGF-R, insulin-like growth factor receptor, Met, myc, p53, BCL-2, ie mutant Ras, mutant p53 a polypeptide associated with the BCR/ABL translocation in CML and ALL; mutant CSF-1 receptor, mutant APC, mutant RET, mutant EGFR, a polypeptide associated with PML/RARA translocation in PML, a polypeptide associated with E2A-PBX1 translocation in pre B leukaemias and in childhood

acute leukaemias, human papilloma virus proteins, Epstein-Barr virus proteins, HTLV-1 proteins, hepatitis B or C virus proteins, herpes-like virus proteins and HIV encoded proteins.

31. A method according to any one of Claims 22 to 30 further comprising determining the HLA class I (or equivalent) type of the healthy individual.

32. A method according to Claim 31 wherein said HLA class I (or equivalent) type is determined by DNA analysis.

33. A method according to any one of Claims 20 to 32 wherein said stimulator cell has a type of HLA class I (or equivalent) molecule on its surface which HLA class I (or equivalent) molecule type is not present in the healthy individual.

34. A method according to any one of Claims 22 to 33 wherein said stimulator cell is a cell which is substantially incapable of loading said HLA class I (or equivalent) molecule with at least a part of said selected molecule.

35. A method according to Claim 34 wherein said cell is a mammalian cell defective in the expression of a peptide transporter.

36. A method according to Claim 35 wherein the mammalian cell lacks or has a reduced level of the TAP peptide transporter.

37. A method according to Claim 34 wherein said cell is an insect cell.

38. A method according to Claim 37 wherein said cell is a *Drosophila*

cell.

39. A method according to any one of Claims 22 to 38 wherein the stimulator cell is a host cell transfected with a nucleic acid molecule capable of expressing said HLA class I (or equivalent) molecule.

40. A method according to Claim 39 wherein said host cell before transfection expresses substantially no HLA class I (or equivalent) molecules.

41. A method according to any one of Claims 22 to 40 wherein said stimulator cell expresses a molecule important for T cell costimulation.

42. A method according to Claim 41 wherein the molecule important for T cell costimulation is any of B7.1, B7.2, ICAM-1 and LFA3.

43. A method according to any one of Claims 22 to 42 wherein substantially all said HLA class I (or equivalent) molecules expressed on the surface of said stimulator cell are of the same type.

44. A clonal population of cytotoxic T lymphocytes reactive against a selected molecule obtainable by the method of any one of Claims 22 to 43.

45. A clonal population of cytotoxic T lymphocytes reactive against a selected molecule wherein the said CTL has a high avidity for a cell presenting said selected molecule in a HLA class I (or

equivalent) molecule.

46. A clonal population of cytotoxic T lymphocytes according to Claim 44 or 45 for use in medicine.

47. A pharmaceutical composition comprising a clonal population of cytotoxic T lymphocytes reactive against a selected molecule according to Claim 44 or 45 and a pharmaceutically acceptable carrier.

48. Use of a clonal population of cytotoxic T lymphocytes derived from a healthy individual and reactive against a selected abnormal molecule derived from a diseased cell from a patient with a disease, or a selected molecule derived from a diseased cell from a patient with a disease wherein an abnormally elevated amount of said molecule is present in said diseased cell, in the manufacture of a medicament for treating a patient with the disease wherein said healthy individual has a different HLA type to said patient.

49. A library of CTL clones, said library comprising a plurality of CTL clones derived from individuals with differing HLA class I (or equivalent) molecule type and each said CTL clone recognises a molecule associated with a selected disease.

50. A therapeutic system comprising (a) means to determine the HLA class I (or equivalent) type of a patient to be treated and (b) a library of CTL clones as defined in Claim 49.

51. Any novel method of treatment using cytotoxic T lymphocytes as herein disclosed.

ABSTRACT

IMMUNOTHERAPY

A method of treating a patient with a disease wherein the patient contains diseased cells which cells contain, or are associated with, an abnormal molecule or an abnormally elevated amount of a molecule and which cells are capable of presenting at least part of said molecule on their surface by an HLA class I (or equivalent) molecule, the method comprising administering to the patient a therapeutically effective amount of cytotoxic T lymphocytes (CTL) which recognise at least part of said molecule when presented by an HLA class I (or equivalent) molecule on the surface of a cell characterised in that the cytotoxic T lymphocytes are not derived from the patient with a disease.

Preferably, the CTL are derived from an individual which individual does not carry the HLA class I (or equivalent) molecule type which, in the patient, presents at least part of said abnormal molecule, or molecule abnormally elevated, contained in or associated with the diseased cells of said patient.

Figure 1

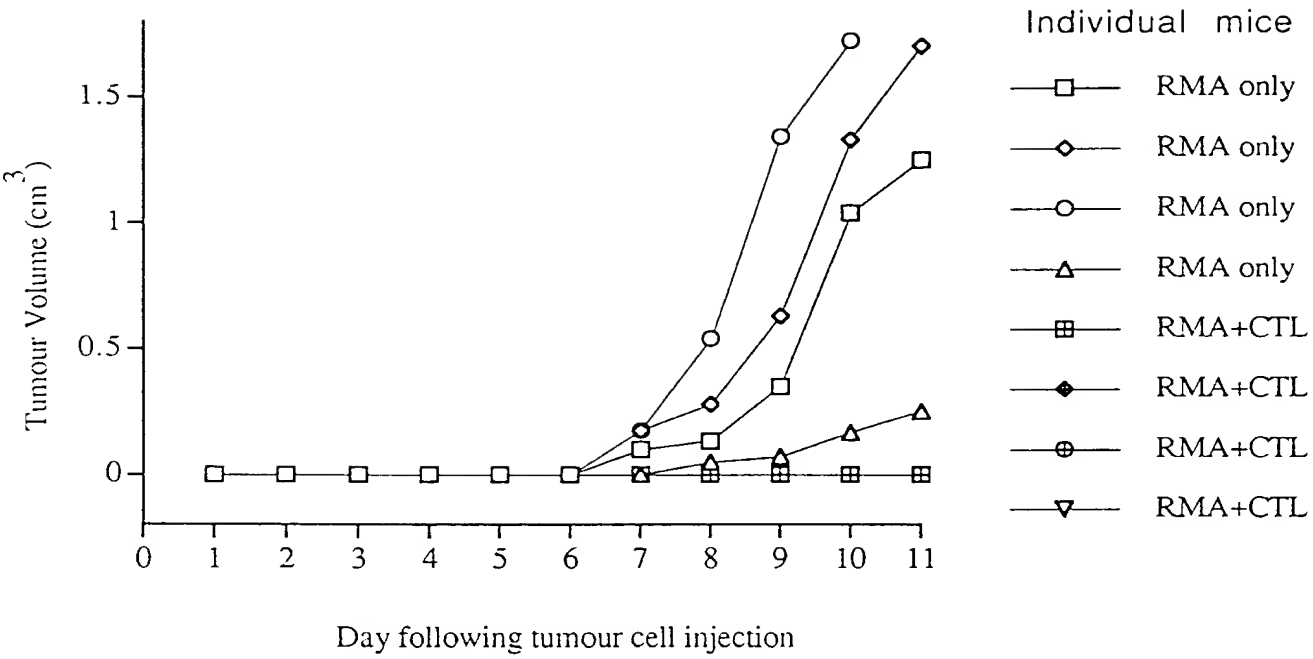

Figure 1: Mice were injected with $5x10^5$ RMA tumour cells only or with tumour cells and $5X10^5$ CTL. The tumour volume was measured every day. After 11 days mice that received tumour cells only were killed because of tumour ulceration or because of large tumour burden. None of the mice that received CTL had detectable tumours at day 11.

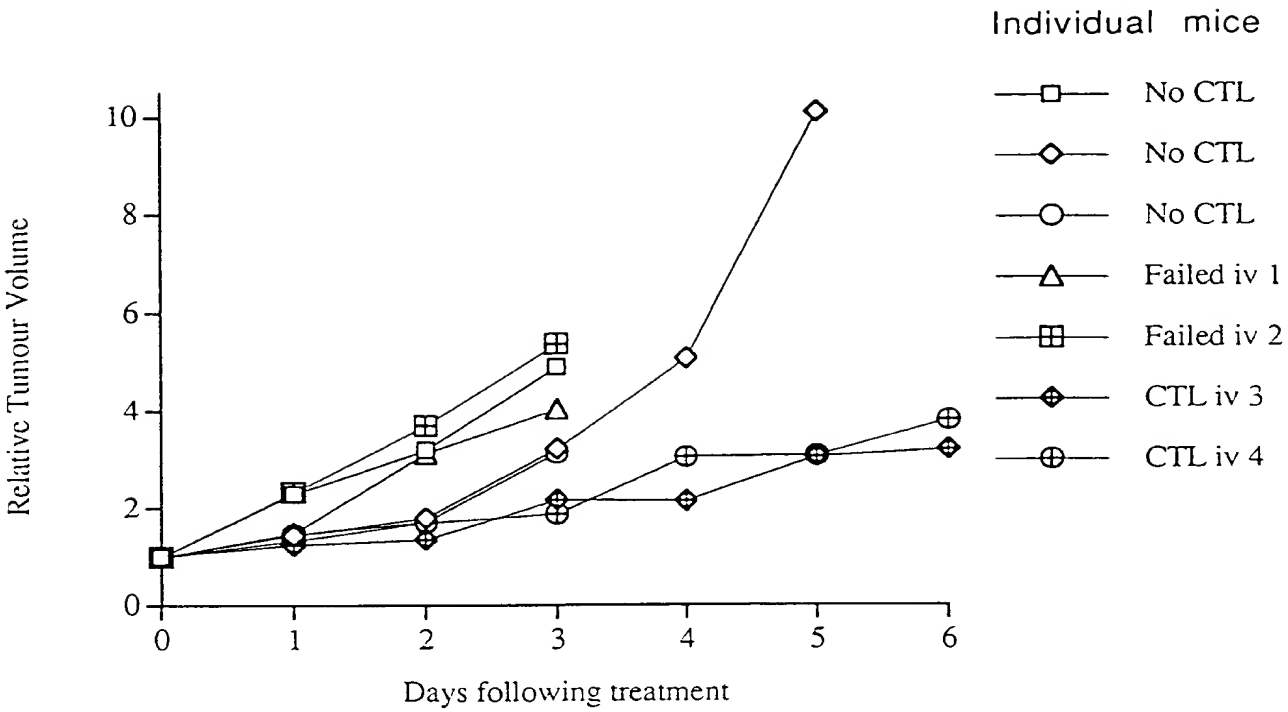

Figure 2: Mice were injected with $5x10^5$ RMA cells at day -7, and at day 0 they were treated with 10^7 anti-mdm100 CTL i.v. The tumour volume was measured at day 0 and each subsequent day. The relative increase in tumour volume is shown. Mice were killed when tumours ulcerated or reached more than 3 cm^3 in volume.

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